(54) Title: A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ABACAVIR SULFATE AND METHODS OF MAK-
ING AND USING SAME

(57) Abstract: A composition comprising a polypeptide and benzatropine mesylate covalently attached to the polypeptide. Also
provided is a method for delivery of benzatropine mesylate to a patient comprising administering to the patient a composition com-
prising a polypeptide and benzatropine mesylate covalently attached to the polypeptide. Also provided is a method for protecting
benzatropine mesylate from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for control-
ling release of benzatropine mesylate from a composition comprising covalently attaching it to the polypeptide.

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ABACAVIR SULFATE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5   The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to abacavir sulfate, as well as methods for protecting and administering abacavir sulfate. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a

10   known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

          Abacavir sulfate is a known pharmaceutical agent – a carbocyclic 2'-

15   deoxyguanosine nucleoside analogue that is a reverse transcriptase inhibitor used in the treatment of HIV. Its chemical name is (1S,4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol. Its structure is as follows:
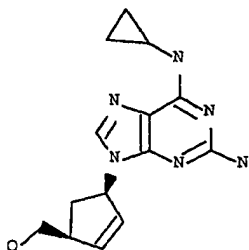
          The novel pharmaceutical compound of the present invention is useful in

20   accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorbtion; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

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compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

5   systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even

10   reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of

15   cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

20   Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release

25   through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

5   microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10   In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral

15   administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.

20   This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the

25   active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

        It is also important to control the molecular weight, molecular size and particle

30   size of the active agent delivery system. Variable molecular weights have unpredictable

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diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

5   Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (abacavir
10   sulfate) to a polymer of peptides or amino acids. The invention is distinguished from the above mentioned technologies by virtue of covalently attaching abacavir sulfate to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
15   conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

20   Alternatively, the present invention provides a pharmaceutical composition comprising abacavir microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and abacavir sulfate covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids,
25   (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

abacavir sulfate preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting abacavir sulfate from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering abacavir sulfate to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, abacavir sulfate is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, abacavir sulfate is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and abacavir sulfate is released from the composition by dissolution of the microencapsulating agent. In another preferred

embodiment, abacavir sulfate is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, abacavir sulfate is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the

5   polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method

10   comprises the steps of:

(a) attaching abacavir sulfate to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

15   (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, abacavir sulfate and a second active agent can be copolymerized in step (c). In

20   another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,

25   cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

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It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000,

5   incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize abacavir sulfate and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of abacavir

10   sulfate. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Abacavir sulfate is the subject of U.S. Patent Numbers 5,034,394 and 5,089,500, herein incorporated by reference, which describes how to make that drug.

15   The composition of the invention comprises abacavir sulfate covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or

20   more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the

25 .   conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

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Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

5   acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van

10   der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant

15   force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of

20   maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the

25   amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

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Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

5   decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other

10   hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15   Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20   Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have

25   profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

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kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond

5   between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and

10   drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be

15   controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the

20   stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

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molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is

5   only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or

10   polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples,

15   the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate

20   with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier.

25   Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will

30   then undergo an intramolecular transamination reaction, thereby, releasing the active

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5   polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10   for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15   distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20   the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25   In the present invention, abacavir sulfate is covalently attached to the polypeptide via its alcohol group or, alternatively, its amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

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Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG)

5   and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal

10   epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the

15   transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active

20   agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the

25   catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-abacavir sulfate conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

5   An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10   **Amine/C-terminus conjugation**

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether

15   and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with

20   triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of

25   other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

5   There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

10   The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

15   **Preparation of γ-Alkyl Glutamate-NCA**

      γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20   **Preparation of Poly[γ-Alkyl Glutamate]**

      γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25   Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

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various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

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CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:

a polypeptide; and

5   abacavir sulfate covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of

10   two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

15   7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein abacavir sulfate is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

20   10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein abacavir sulfate is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing abacavir sulfate from said composition in a pH-dependent manner.

19. A method for protecting abacavir sulfate from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of abacavir sulfate from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching abacavir sulfate to said polypeptide.

21. A method for delivering abacavir sulfate to a patient comprising administering to said patient a composition comprising:
    a polypeptide; and
    abacavir sulfate covalently attached to said polypeptide.

22. The method of claim 21 wherein abacavir sulfate is released from said composition by an enzyme-catalyzed release.

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23. The method of claim 21 wherein abacavir sulfate is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5   25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10   Abstract

A composition comprising a polypeptide and abacavir sulfate covalently attached to the polypeptide. Also provided is a method for delivery of abacavir sulfate to a patient comprising administering to the patient a composition comprising a polypeptide and abacavir sulfate covalently attached to the polypeptide. Also provided is a method for
15   protecting abacavir sulfate from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of abacavir sulfate from a composition comprising covalently attaching it to a polypeptide.

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ABARELIX AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to abarelix, as well as methods for protecting and administering abarelix. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Abarelix is a known pharmaceutical agent that is used in the treatment of prostate cancer, acting as a gonadotropin-releasing hormone antagonist. Its chemical name is N-acetyl-3-(2-naphthalenyl)-D-alanyl-4-chloro-D-phenylalanyl-3-(3-pyridinyl)-D-alanyl-L-seryl-N-methyl-L-tyrosyl-D-asparagynyl-L-N6-(1-methylethyl)-L-lysyl-L-prolyl-D-alaninamide. Abarelix is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is as follows:

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The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorbtion; targeted delivery to particular tissue/cell type;

5   and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

10   systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even

15   reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of

20   cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

25   Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release

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through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

5   Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

10   microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

15   In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral

20   administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.

25   This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the

30   active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide

linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle

5   size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

10 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (abarelix)

15 to a polymer of peptides or amino acids. The invention is distinguished from the above mentioned technologies by virtue of covalently attaching abarelix to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection.

20 In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

25 Alternatively, the present invention provides a pharmaceutical composition comprising abarelix microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and abarelix covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,

(ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a

heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a

synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a

heteropolymer of one or more naturally occurring amino acids and one or more synthetic

5   amino acids.

        Abarelix preferably is covalently attached to a side chain, the N-terminus or the

C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In

another preferred embodiment, the active agent is an amine and is covalently attached to

10   the C-terminus of the polypeptide. In another preferred embodiment, the active agent is

an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another

preferred embodiment, the active agent is an alcohol and is covalently attached to the N-

terminus of the polypeptide.

        The composition of the invention can also include one or more of a

15   microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The

microencapsulating agent can be selected from polyethylene glycol (PEG), an amino

acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant

preferably activates an intestinal transporter.

        Preferably, the composition of the invention is in the form of an ingestable tablet,

20   an intravenous preparation or an oral suspension. The active agent can be

conformationally protected by folding of the polypeptide about the active agent. In

another embodiment, the polypeptide is capable of releasing the active agent from the

composition in a pH-dependent manner.

        The invention also provides a method for protecting abarelix from degradation

25   comprising covalently attaching it to a polypeptide.

        The invention also provides a method for delivering abarelix to a patient, the

patient being a human or a non-human animal, comprising administering to the patient a

composition comprising a polypeptide and an active agent covalently attached to the

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polypeptide. In a preferred embodiment, abarelix is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, abarelix is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a

5   microencapsulating agent and abarelix is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, abarelix is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, abarelix is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant

10   covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

        The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method

15   comprises the steps of:

        (a) attaching abarelix to a side chain of an amino acid to form an active agent/amino acid complex;

        (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

20   (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

        In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, abarelix and a second active agent can be copolymerized in step (c). In another

25   preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,

30   cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a

carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

5   It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

10   The present invention provides several benefits for active agent delivery. First, the invention can stabilize abarelix and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of abarelix. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted

15   delivery of active agents to specifics sites of action.

        The composition of the invention comprises abarelix covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a

20   heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

        Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and

25   turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

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Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

5   acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van

10   der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant

15   force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of

20   maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the

25   amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

5   decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other

10   hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15   Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20   Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have

25   profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond

5   between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and

10   drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be

15   controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B$_6$ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B$_2$ (Riboflavin)	376
		Vitamin D$_2$	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the

20   stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

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molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

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agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5   polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10   for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15   distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20   the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25   In the present invention, abarelix is covalently attached to the polypeptide via the free alcohol group or, alternatively, through one of its amino groups.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

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Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG)

5   and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal

10   epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the

15   transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active

20   agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the

25   catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-abarelix conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 5   0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10   **Amine/C-terminus conjugation**

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether 15   and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with 20   triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of 25   other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

5   There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

10   The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

15   **Preparation of γ-Alkyl Glutamate-NCA**

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20   **Preparation of Poly[γ-Alkyl Glutamate]**

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25   Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

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various modifications may be made in the details within the scope and range of
equivalents of the claims and without departing from the spirit of the invention.

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CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:

a polypeptide; and

5   abarelix covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of

10   two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

15   7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein abarelix is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

20   10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5   14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an
10   oral suspension.

17. The composition of claim 1 wherein abarelix is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing abarelix from said composition in a pH-dependent manner.

15   19. A method for protecting abarelix from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of abarelix from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching abarelix to said polypeptide.

20   21. A method for delivering abarelix to a patient comprising administering to said patient a composition comprising:
       a polypeptide; and
       abarelix covalently attached to said polypeptide.

-      22. The method of claim 21 wherein abarelix is released from said composition
25   by an enzyme-catalyzed release.

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23. The method of claim 21 wherein abarelix is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5   25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

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Abstract

A composition comprising a polypeptide and abarelix covalently attached to the polypeptide. Also provided is a method for delivery of abarelix to a patient comprising administering to the patient a composition comprising a polypeptide and abarelix

15   covalently attached to the polypeptide. Also provided is a method for protecting abarelix from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of abarelix from a composition comprising covalently attaching it to the polypeptide.

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A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ACARBOSE AND METHODS OF MAKING AND USING SAME
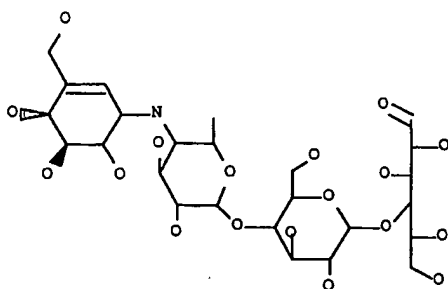
FIELD OF THE INVENTION

5   The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to acarbose, as well as methods for protecting and administering acarbose. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of

10   the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

         Acarbose is a known pharmaceutical agent that is used in the treatment of type II

15   diabetes. Its chemical name is O-4,6-dideoxy-4-[[[1S-(1alpha,4alpha,5beta,6alpha)]-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino]-alpha-D-glucopyranosyl-(1-4)-O-alpha-D-glucopyranosyl-(1-4)-D-glucose. Its structure is as follows:

20   The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorbtion; targeted delivery to particular tissue/cell type;

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and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

5   Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf

10   life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as

15   resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the

20   stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human

25   growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

5 reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

10 unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active

15 agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide

20 spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders

25 and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group

30 with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
size of the active agent delivery system. Variable molecular weights have unpredictable
diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly
or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in
5   the colon by bacterial enzymes. High molecular weight microspheres usually have high
moisture content which may present a problem with water labile active ingredients.
Particle size not only becomes a problem with injectable drugs, as in the HAR
application, but absorption through the brush-border membrane of the intestines is limited
to less than 5 microns.

10   SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (acarbose)
to a polymer of peptides or amino acids. The invention is distinguished from the above
mentioned technologies by virtue of covalently attaching acarbose to the N-terminus, the
C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide,
15   also referred to herein as a carrier peptide. In certain applications, the polypeptide will
stabilize the active agent, primarily in the stomach, through conformational protection.
In these applications, delivery of the active agent is controlled, in part, by the kinetics of
unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous
enzymes release the active ingredient for absorption by the body by selectively
20   hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a
second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition
comprising acarbose microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and acarbose
25   covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
(ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a
synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a

heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

acarbose preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

5   carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-

10 terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant

15 preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the

20 composition in a pH-dependent manner.

The invention also provides a method for protecting acarbose from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering acarbose to a patient, the patient being a human or a non-human animal, comprising administering to the patient a

25 composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, acarbose is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, acarbose is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release.

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In another preferred embodiment, the composition further comprises a microencapsulating agent and acarbose is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, acarbose is released from the composition by a pH-dependent unfolding of the polypeptide. In another

5 preferred embodiment, acarbose is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

10 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching acarbose to a side chain of an amino acid to form an active agent/amino acid complex;

15 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a

20 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, acarbose and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular

25 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the

30 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize acarbose and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of acarbose. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Acarbose is the subject of U.S. Patent Number 4,904,769, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises acarbose covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

5   acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van

10   der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant

15   force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of

20   maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the

25   amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond

5   between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and

10   drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be

15   controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the

20   stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

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agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, acarbose is covalently attached to the polypeptide via any of the free hydroxyl groups.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG)

5   and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal

10   epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the

15   transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active

20   agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the

25   catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-acarbose conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to
5   0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10   **Amine/C-terminus conjugation**

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether
15   and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with
20   triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of
25   other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

5   There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

10   The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

15   **Preparation of γ-Alkyl Glutamate-NCA**

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20   **Preparation of Poly[γ-Alkyl Glutamate]**

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25   Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of
equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
acarbose covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein acarbose is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5   14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an
10   oral suspension.

17. The composition of claim 1 wherein acarbose is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing acarbose from said composition in a pH-dependent manner.

15   19. A method for protecting acarbose from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of acarbose from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching acarbose to said polypeptide.

20   21. A method for delivering acarbose to a patient comprising administering to said patient a composition comprising:
           a polypeptide; and
           acarbose covalently attached to said polypeptide.

22. The method of claim 21 wherein acarbose is released from said composition
25   by an enzyme-catalyzed release.

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23. The method of claim 21 wherein acarbose is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5   25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10

Abstract

        A composition comprising a polypeptide and acarbose covalently attached to the
15   polypeptide. Also provided is a method for delivery of acarbose to a patient comprising administering to the patient a composition comprising a polypeptide and acarbose covalently attached to the polypeptide. Also provided is a method for protecting acarbose from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of acarbose from a composition comprising covalently
20   attaching it to the polypeptide.

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
ACETAMINOPHEN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5   The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to acetaminophen, as well as methods for protecting and administering acetaminophen. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of

10   the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

         Acetaminophen is a known pharmaceutical agent that is used in the treatment of

15   minor aches and pains.   Its chemical name is N-acetyl-p-aminophenol. Acetaminophen is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art.

         The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability

20   of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorbtion; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25   Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another

invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

5   Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the

10   active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and

15   aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can

20   also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

25   reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically
5   require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the
10   gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The
15   released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that
20   incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly
25   or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited
30   to less than 5 microns.

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SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (acetaminophen) to a polymer of peptides or amino acids. The invention is distinguished from the above mentioned technologies by virtue of covalently attaching acetaminophen to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising acetaminophen microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and acetaminophen covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

acetaminophen preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

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The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting acetaminophen from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering acetaminophen to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, acetaminophen is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, acetaminophen is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and acetaminophen is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, acetaminophen is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, acetaminophen is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching acetaminophen to a side chain of an amino acid to form an active

5   agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

10   In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, acetaminophen and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and

15   wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a

20   carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

25   The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First,

30   the invention can stabilize acetaminophen and prevent its digestion in the stomach. In

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addition, the pharmacologic effect can be prolonged by delayed release of acetaminophen. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

5   The composition of the invention comprises acetaminophen covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or

10   more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the

15   conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding

20   are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best

25   model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior

and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will

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ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
5   be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
10   active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
15   length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
20   active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate
25   weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the

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jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

5   TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For

10   example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could

15   conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-

20   terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order

to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example

5   above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

10   The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal

15   enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

20   carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart

25   specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these

30   polypeptides through a spacer or linker on the pendant group, which is terminated,

preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the

5   alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-

10   dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, acetaminophen is covalently attached to the polypeptide via its hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A

15   preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG)

20   and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal

25   epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known

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intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,

5   sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is

10   particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

15   Preferably, the resultant peptide-acetaminophen conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

20   An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{\circ}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or

25   dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

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The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{\circ}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

5   precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product,

10   which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and

15   filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

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CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
acetaminophen covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein acetaminophen is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

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12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5   14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an 10   oral suspension.

17. The composition of claim 1 wherein acetaminophen is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing acetaminophen from said composition in a pH-dependent manner.

15   19. A method for protecting acetaminophen from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of acetaminophen from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching acetaminophen to said polypeptide.

20   21. A method for delivering acetaminophen to a patient comprising administering to said patient a composition comprising:
        a polypeptide; and
        acetaminophen covalently attached to said polypeptide.

22. The method of claim 21 wherein acetaminophen is released from said 25   composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein acetaminophen is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5   25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

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A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ACETAMINOPHEN AND CODEINE AND METHODS OF MAKING AND USING SAME

5   FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to both acetaminophen and codeine, as well as methods for protecting and administering acetaminophen and codeine together. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA),

10   has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15   Acetaminophen is a known pharmaceutical agent that is used in the treatment of minor aches and pains. Its chemical name is N-acetyl-p-aminophenol. It is often used in combination with codeine, whose chemical name is 7,8-didehydro-4,5-α-epoxy-3-methoxy-17-methylmephorninan-6α-ol. Both are commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art.

20   The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorbtion; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

25   compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken

under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even

5   reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of

10 cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

15 Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release

20 through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

25 shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent

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in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

5   In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral

10   administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.

15   This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the

20   active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

         It is also important to control the molecular weight, molecular size and particle

25   size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

30   Particle size not only becomes a problem with injectable drugs, as in the HAR

application, but absorption through the brush-border membrane of the intestines is limited
to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent
5   (acetaminophen and codeine) to a polymer of peptides or amino acids. The invention is
distinguished from the above mentioned technologies by virtue of covalently attaching
acetaminophen and codeine to the N-terminus, the C-terminus or directly to the amino
acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier
peptide. In certain applications, the polypeptide will stabilize the active agent, primarily
10   in the stomach, through conformational protection. In these applications, delivery of the
active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide.
Upon entry into the upper intestinal tract, indigenous enzymes release the active
ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the
carrier peptide. This enzymatic action introduces a second order sustained release
15   mechanism.

Alternatively, the present invention provides a pharmaceutical composition
comprising acetaminophen and codeine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and
acetaminophen and codeine covalently attached to the polypeptide. Preferably, the
20   polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally
occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino
acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more
synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino
acids and one or more synthetic amino acids.

25   acetaminophen and codeine preferably is covalently attached to a side chain, the
N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active
agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide.
In another preferred embodiment, the active agent is an amine and is covalently attached

to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5   The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10   Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15   The invention also provides a method for protecting acetaminophen and codeine from degradation comprising covalently attaching it to a polypeptide.

        The invention also provides a method for delivering acetaminophen and codeine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently

20   attached to the polypeptide. In a preferred embodiment, acetaminophen and codeine are released from the composition by an enzyme-catalyzed release. In another preferred embodiment, acetaminophen and codeine are released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and

25   acetaminophen and codeine are released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, acetaminophen and codeine are released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, acetaminophen and codeine are released from the composition in a sustained release. In yet another preferred embodiment, the

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composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5   The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching acetaminophen to a side chain of an amino acid to form an active agent/amino acid complex;

10   (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a

15   second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, acetaminophen and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular

20   transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the

25   glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First,
5   the invention can stabilize acetaminophen and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of acetaminophen. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

10   The composition of the invention comprises acetaminophen covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15   more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20   conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25   are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

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Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5   Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the 10   peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, 15   aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight 20   active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the 25   carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and

5   drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be

10   controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the

15   stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

20   delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently
attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or
5   polypeptide. The location of attachment depends somewhat on the functional group
selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order
to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples,
10   the peptide is, in essence, extended by one monomeric unit forming a new peptide bond.
If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
preferred point of attachment in order to achieve a stable composition. As in the example
above where the alcohol, norethindrone, was covalently attached to
poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate
15   with phosgene. This invention, then, pertains to the reaction of this key intermediate
with the N-terminus of the peptide carrier. The active ingredient can be released from
the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid
and then this conjugate covalently attached to the C-terminus of the peptide carrier.
20   Because the glutamic acid-drug conjugate can be considered a dimer, this product adds
two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety
from the peptide carrier. The newly formed free amine of the glutamic acid residue will
25   then undergo an intramolecular transamination reaction, thereby, releasing the active
agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
carboxyanhydride. This intermediate can then be polymerized, as described above, using
any suitable initiator as shown in Fig. 4. The product of this polymerization is
30   polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

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maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, acetaminophen and codeine are covalently attached to the polypeptide via its hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

5   There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the

10   transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active

15   agents.

       In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the

20   catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

       Preferably, the resultant peptide-acetaminophen conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

25   Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be
5   stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
10   The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

15   In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product
20   is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include
25   dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

5   several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole

10   followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and

15   the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of

20   a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

25   various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
acetaminophen covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein acetaminophen and codeine are covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5   14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an
10   oral suspension.

17. The composition of claim 1 wherein acetaminophen and codeine are conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing acetaminophen from said composition in a pH-dependent manner.

15   19. A method for protecting acetaminophen from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of acetaminophen from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching acetaminophen to said polypeptide.

20   21. A method for delivering acetaminophen to a patient comprising administering to said patient a composition comprising:
        a polypeptide; and
        acetaminophen covalently attached to said polypeptide.

22. The method of claim 21 wherein acetaminophen and codeine are released
25   from said composition by an enzyme-catalyzed release.

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23. The method of claim 21 wherein acetaminophen and codeine are released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5   25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

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Abstract

A composition comprising a polypeptide and acetaminophen covalently attached to the polypeptide. Also provided is a method for delivery of acetaminophen to a patient comprising administering to the patient a composition comprising a polypeptide and acetaminophen covalently attached to the polypeptide. Also provided is a method for protecting acetaminophen from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of acetaminophen from a composition comprising covalently attaching it to the polypeptide.

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A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
PROPOXYPHENE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5   The present invention relates to a novel pharmaceutical compound that comprises

a polypeptide that is preferably covalently attached to propoxyphene, as well as methods

for protecting and administering propoxyphene. This novel compound, referred to as a

CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known

effective pharmaceutical agent that is both well studied and occupies a known segment of

10   the pharmaceutical market, and combining it with a carrier compound that enhances the

usefulness of the pharmaceutical agent without compromising its pharmaceutical

effectiveness.

BACKGROUND OF THE INVENTION

        Propoxyphene is a known pharmaceutical agent that is used in the treatment of

15   pain. It is a mild narcotic analgesic. It is both commercially available and readily

manufactured using published synthetic schemes by those of ordinary skill in the art. Its

structure is as follows:
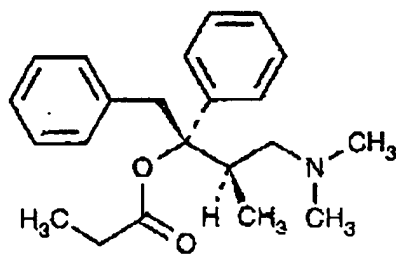
        The novel pharmaceutical compound of the present invention is useful in

20   accomplishing one or more of the following goals: enhancement of the chemical stability

of the original compound; alteration of the release profile of an orally administered

product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;

and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical

25   agent, an adjuvant, or an inhibitor.

1

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

5   markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly

10   acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the

15   active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and

20   aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can

25   also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some

5   technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically

10   require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the

15   gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The

20   released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that

25   incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly

30   or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high

moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR

application, but absorption through the brush-border membrane of the intestines is limited

5   to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent

(propoxyphene) to a polymer of peptides or amino acids. The invention is distinguished

from the above mentioned technologies by virtue of covalently attaching propoxyphene

10   to the N-terminus, the C-terminus or directly to the amino acid side chain of an

oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain

applications, the polypeptide will stabilize the active agent, primarily in the stomach,

through conformational protection. In these applications, delivery of the active agent is

controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the

15   upper intestinal tract, indigenous enzymes release the active ingredient for absorption by

the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This

enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition

comprising propoxyphene microencapsulated by a polypeptide.

20   The invention provides a composition comprising a polypeptide and

propoxyphene covalently attached to the polypeptide. Preferably, the polypeptide is (i)

an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids,

(iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer

of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or

25   (vi) a heteropolymer of one or more naturally occurring amino acids and one or more

synthetic amino acids.

propoxyphene preferably is covalently attached to a side chain, the N-terminus or

the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another

5   preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino

10 acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In

15 another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting propoxyphene from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering propoxyphene to a patient,

20 the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, propoxyphene is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, propoxyphene is released in a time-dependent manner based on the pharmacokinetics of

25 the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and propoxyphene is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, propoxyphene is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, propoxyphene is

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released from the composition in a sustained release. In yet another preferred
embodiment, the composition further comprises an adjuvant covalently attached to the
polypeptide and release of the adjuvant from the composition is controlled by the
polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug

5   conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a
polypeptide and an active agent covalently attached to the polypeptide. The method
comprises the steps of:

(a) attaching propoxyphene to a side chain of an amino acid to form an active

10   agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA)
from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride
(NCA).

15   In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
second active agent. When steps (a) and (b) are repeated prior to step (c) with a second
agent, propoxyphene and a second active agent can be copolymerized in step (c). In
another preferred embodiment, the amino acid is glutamic acid and the active agent is
released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and

20   wherein the active agent is released from the glutamic acid by coincident intramolecular
transamination. In another preferred embodiment, the glutamic acid is replaced by an
amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side
chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a

25   carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an
amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the
following detailed description are exemplary, but are not restrictive, of the invention.

30   The general applications of this invention to other active pharmaceutical agents is

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described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5   The present invention provides several benefits for active agent delivery. First, the invention can stabilize propoxyphene and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of propoxyphene. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

10   The composition of the invention comprises propoxyphene covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15   more naturally occurring amino acids and one or more synthetic amino acids.

        Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20   conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

        Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25   are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational

5   entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular

10   bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has

15   maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is

20   a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious

25   chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5   Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the
10   peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine,
15   aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight
20   active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the
25   carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and

5   drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be

10   controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the

15   stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

20   delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or
5   polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples,
10   the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate
15   with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier.
20   Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will
25   then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is
30   polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5   for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10   distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15   the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20   In the present invention, propoxyphene is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A
25   preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

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The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-propoxyphene conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to
$0^{\circ}C$. The solution can then be treated with diisopropylcarbodiimide and
hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be
5   stirred for several hours at room temperature, the urea by-product filtered off, the product
precipitated out in ether and purified using gel permeation chromatography (GPC) or
dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{\circ}C$.
10   The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
followed by the amine bioactive agent. The reaction can then be stirred for several hours
at room temperature, the urea by-product filtered off, the product precipitated out in ether
and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

15   In the following example the combination of the alcohol with triphosgene
produces a chloroformate, which when reacted with the N-terminus of the peptide
produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with
triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then
added slowly and the solution stirred at room temperature for several hours. The product
20   is then precipitated out in ether. The crude product is suitably deprotected and purified
using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of
other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated
solvents such as chloroform. Examples of other activating agents include
25   dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-
hydroxysuccinimide. Examples of bases include pyrrolidinopyridine,
dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5   several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.   .

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{\circ}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10   followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15   the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20   a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25   various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

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<u>CLAIMS</u>

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
propoxyphene covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein propoxyphene is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein propoxyphene is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing propoxyphene from said composition in a pH-dependent manner.

19. A method for protecting propoxyphene from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of propoxyphene from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching propoxyphene to said polypeptide.

21. A method for delivering propoxyphene to a patient comprising administering to said patient a composition comprising:
    a polypeptide; and
    propoxyphene covalently attached to said polypeptide.

22. The method of claim 21 wherein propoxyphene is released from said composition by an enzyme-catalyzed release.

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23. The method of claim 21 wherein propoxyphene is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5   25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

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Abstract

A composition comprising a polypeptide and propoxyphene covalently attached to the polypeptide. Also provided is a method for delivery of propoxyphene to a patient comprising administering to the patient a composition comprising a polypeptide and propoxyphene covalently attached to the polypeptide. Also provided is a method for protecting propoxyphene from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of propoxyphene from a composition comprising covalently attaching it to the polypeptide.